Patent claims

1. Method for identifying and quantifying tumour-associated peptides, comprising the steps:
providing a first sample of tissue or cells,
providing a second sample of tissue or cells with the identical amount by weight or cellular count as the first sample,
obtaining peptides from the first and the second sample,
separately, chemically identically modifying the peptides from both samples in order to generate different physical characteristics in the peptides from the different samples,
mixing of the so modified peptides from both samples,
determining the amino acid sequences of the peptides, and
determining the relative amount ratios of peptides from both samples having identical sequences, based on the different physical characteristics.

2. Method according to claim 1, characterised in that the peptides from both samples are chemically modified by using at least two different stable isotopes of the same element.

3. Method according to claim 1 or 2, characterised by the following steps:
   a) providing a sample from tumourous and a sample from corresponding healthy tissue or accordingly transfected or infected cell lines, wherein both samples have identical amounts per weight or cellular counts,
   b) isolating peptides from said sample from tumourous tissue,
   c) isolating peptides from said sample from corresponding healthy tissue,
   d) chemically modifying the peptides obtained in step (b) with a chemical group that contains a stable isotope of an element from the periodic system of the elements,
   e) chemically modifying the peptides obtained in step (c) with a chemical group that contains a second stable isotope of the element from the periodic system of the elements used in step d),
   f) mixing of the chemically modified peptides obtained from steps (d) and (e),
   g) separating the peptides obtained from step f) by a chromatographic method,

h) identifying and determining peptides having identical amino acid sequences and the ratio by weight of chemically modified peptides with identical amino acid sequences from step (g),

i) identifying tumour-associated peptides that are suitable, preferably particularly suitable for the combination into a therapeutic vaccine, based on the data obtained from step (h).

4. Method according to any of claims 1 to 3, characterised in that the tumour-associated peptides are MHC-class I-ligands.

5. Method according to claim 3 or 4, characterised in that in step d) deuterium ($^2$D) and in step e) regular hydrogen ($^1$H) are used as stable isotopes.

6. Method according to any of claims 3 to 5, characterised in that in step g) a chromatographic separation of the peptides by HPLC is performed.

7. Method according to any of claims 3 to 6, characterised in that step h) is performed by mass spectrometric analysis.

8. Method according to claim 7, characterised in that the mass spectrometric analysis for determining the amount ratios of the two peptides with identical amino acid sequence and identical chemical modification is performed based on at least two different isotopes of the same element from the periodic system of elements.

9. Method according to claim 8, characterised in that the relative intensity of the signals as measured for peptides having identical amino acid sequence and identical chemical modification but different isotopes of the same element that are simultaneously present is used for calculating the relative quantitative ratio between said peptides.

10. Method according to any of claims 3 to 9, characterised in that in step i) suitable databases for the identification of tumour-associated genes and gene products are used.

11. Method according to any of claims 3 to 10, characterised in that following step h) an additional step is performed, wherein the reactivity of peripheral leukocytes, preferably of T-lymphocytes, against the identified and quantified peptides is tested.

12. Method according to claim 11, characterised in that the testing of the reactivity takes place by measuring the cytokine-mRNA and/or interferon-mRNA that is synthesised by the leukocytes.

13. Method according to claim 11, characterised in that the testing of the reactivity takes place by the activation of peripheral T-lymphocytes by means of reconstituted complexes from antigen-presenting molecules and peptides.

14. Method according to claim 13, characterised in that the complexes of antigen-presenting molecules and peptides that are used for activating T-lymphocytes are fixed on a surface.

15. Method according to claim 14, characterised in that the surface that is used for fixing said complexes of antigen-presenting molecules and peptides consists of polystyrol.

16. Method according to any of claims 13 to 15, characterised in that the antigen presenting molecules are coupled by chemical reaction with biotin, and the surface that is used is made from polystyrol being coated with streptavidin by a chemical reaction.

17. Method for producing a peptide, wherein a peptide is identified according to the method according to any of claims 1 to 16, and the identified peptide is synthesised chemically, in vitro or in vivo.

18. Peptide identified according to a method according to any of claims 1 to 16 and/or produced according to a method according to claim 17.

19. Tumour-associated peptide having an amino acid sequence that is selected from the group consisting of SEQ ID-No. 1 to 36 from the accompanying sequence protocol,

wherein said peptide has the ability to bind to a molecule of the human major histocompatibility complex (MHC) class-I.

20. Peptide according to claim 18 or 19, characterised in that at least one amino acid is replaced by another amino acid having similar chemical characteristics.

21. Peptide according to any of claims 18 to 20, characterised in that at least one additional amino acid is present at the N- or C-terminus.

22. Peptide according to any of claims 18 to 21, characterised in that at least one amino acid is deleted.

23. Peptide according to any of claims 18 to 22, characterised in that at least one amino acid is chemically modified.

24. Use of one or several of the peptides according to any of claims 18 to 23 for producing a medicament for the treatment of tumourous diseases and/or adenomatous diseases.

25. Use of the peptide according to any of claims 18 to 23 for the treatment of tumourous diseases and/or adenomatous diseases.

26. Use according to claim 24 or 25, characterised in that the disease is renal, lung, colon, stomach, pancreatic, breast, prostate, ovarian and/or skin cancer.

27. Use according to any of claims 24 to 26, characterised in that the peptide is used together with an adjuvans.

28. Use according to any of claims 24 to 27, characterised in that a peptide bound to an antigen presenting cell is used.

29. Use of the peptide according to any of claims 18 to 23 for the labelling of leukocytes, in particular of T-lymphocytes.

30. Use of the peptide according to any of claims 18 to 23 for evaluating the progress of therapy in a tumourous disease.

31. Use of the peptide according to any of claims 18 to 23 for producing an antibody.

32. Pharmaceutical composition, containing one or several peptides according to any of claims 18 to 23.

33. Nucleic acid molecule, encoding the peptide according to any of claims 18 to 23.

34. Vector, comprising the nucleic acid molecule according to claim 33.

35. Cell that was genetically modified with the aid of the nucleic acid molecule according to claim 33 or with the vector according to claim 34 in such a manner that it expresses a peptide according to any of claims 18 to 23.

36. Use of the nucleic acid molecule from claim 33 and/or the vector from claim 34 and/or the cell from claim 35 for producing a medicament for the treatment of tumourous diseases and/or adenomatous diseases.

37. Method for producing a vaccine with the steps:
    a) performing the methods according to any of claims 1 to 16,
    b) producing the identified peptides, and
    c) formulating the produced peptides into the vaccine.

38. Diagnostic method, wherein the method according to any of claims 1 to 16 is performed, and the presence and/or the amount ratio of a peptide is used as a diagnostic marker.

39. Method for treatment of a pathological condition, wherein an immune response against a protein of interest is triggered, characterised in that a therapeutic effective amount of at least one of the peptides according to any of claims 18 to 23 is administered.

40. Electronic storage medium containing the amino acid sequence of at least one of the peptides according to any of claims 18 to 23 and/or the nucleic acid sequence of the nucleic acid molecule according to claim 33.